

# Interaction Between Photoperiod and an Endogenous Seasonal Factor in Influencing the Diel Locomotor Activity of the Benthic Polychaete *Nereis virens* Sars

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**Abstract.** The locomotor activity of *Nereis virens* Sars associated with food prospecting was investigated in response to photoperiod and season using an actograph. Experimental animals which had been reared under natural photoperiods were exposed to two constant photoperiodic treatments, LD 16:8 and LD 8:16, in both the autumn and winter and in the absence of tidal entrainment. Autocorrelation analysis of rhythmicity showed that during the autumn, animals under the LD 16:8 photoperiod displayed a strong nocturnal rhythm of activity, whereas animals under the LD 8:16 photoperiod showed only a weak nocturnal activity rhythm. This is believed to represent an autumn feeding cessation that is triggered when the animals pass through a critical photoperiod  $LD_{crit} <12;>12$ . Later in the winter, however, animals exposed to both photoperiodic treatments showed strong rhythms of foraging activity irrespective of the imposed photoperiod. It is suggested that the autumn cessation may maximize the fitness of *N. virens*, a spring-breeding semelparous organism, by reducing risk during gamete maturation, while spontaneous resurgence of activity after the winter solstice permits animals that are not physiologically competent to spawn to accrue further metabolic reserves. This response is believed to be initiated by a seasonal (possibly circannual) endogenous oscillator or interval timer.

## Introduction

*Nereis virens* Sars, one of the largest marine annelids, has proven to be an interesting model for studies of semelparous

life histories with mixed age at maturity (Olive *et al.*, 1997, 2000, 2001). While age at maturity varies between 1 and 7 or more years, any one worm can breed only once, whereupon spawning is followed by death. A key transition in this life history occurs during the autumn preceding eventual breeding when the final stages of gametogenesis are initiated, culminating in individuals becoming gravid and spawning during the following spring. The profound influence of the photoperiod on the behavior and physiology of *N. virens* has already been demonstrated. Transition through the critical photoperiod ( $LD_{crit} <12;>12$ ) at the end of September (Last *et al.*, 1999) initiates sexual maturation and gamete development, which may culminate in sexual reproduction and death in any particular year (Olive *et al.*, 1997). When semiquantitative methods were used to determine the prospecting behavior of this polychaete, it was shown (Last and Olive, 1999) that the frequency at which animals emerged from their burrows in the autumn was much higher under a photoperiod of LD (light/dark) 16:8 than under LD 8:16. In a potentially long-lived but strictly semelparous organism such as *N. virens*, there is strong selection for the avoidance of risk during the final stages of sexual maturation when accumulated reserves are being converted to gametogenic tissues (Olive *et al.*, 2001). It is reasonable, therefore, to interpret the level of foraging activity as indicating the onset of a physiological state, responsive to relative daylength, during which sexual maturation occurs. Observations made during the late spring and summer, however (Last and Olive, 1999), showed that the differences in foraging activity between LD 16:8 and LD 8:16 were not as marked, but were still significantly different. It was therefore suggested that a covert temporal rhythm must underlie the overt activity of emergence in *N. virens*, and that the emergent patterns also involve expression of a

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response to a seasonal (or circannual) clock, since exposure to a constant LD 8:16 photoperiod does not induce the same physiological response at all times of the year.

Photoperiodism in marine animals is less intensively studied than in terrestrial organisms, but "photoperiod-like" phenomena have been documented in several marine species, including the echinoderm *Picaster ochraceus* (Pearse and Eernisse, 1982), the copepod *Labidocera aestiva* (Marcus, 1986), and the polychaetes *Neanthes limicola* (Fong and Pearce, 1992a, b) and *Harmothoe imbicata* (Garwood and Olive, 1982). The difference between the environmental and the evolutionary history in marine organisms requires that these processes be investigated to understand environmental signal transduction in the control of life-history events and to determine whether these observations are likely to be the consequence of a common clock mechanism. Confirmation that they are such a consequence would have important implications for our understanding of the evolution of clock-based processes.

To test the suggestion that an endogenous long-term rhythm moderates responses to fixed photoperiodic inputs in *N. virens*, we used an actographic procedure (Last, 2003) to monitor its emergence (foraging) behavior and examined the spontaneous diel activity patterns of individual animals maintained under fixed LD cycles for several months.

### Materials and Methods

Two experiments were conducted using the polychaete *Nereis virens*. Short-term assays of activity were carried out in the autumn and winter to assess the strength of diel "out-of-burrow" activity both within and between seasons under rectilinear photoperiods (that is, light and dark periods without simulated dawn and dusk). Concurrently, a long-term experiment was used to determine any spontaneous changes in activity under constant rectilinear photoperiods with time.

Two time periods were used for the short-term assay: six days in September in the autumn of 1998 (A98) and six days in February in the winter of 1999 (W99). Both A98 and W99 animals had been born from the same broodstock (16 March 1998), and as a consequence, the experimental animals for W99 were 5 months older than those used for A98. To prevent any size- or maturation-related bias in feeding rates, all animals chosen were of about the same weight,  $2.5 \pm 0.1$  g, with all coelomic oocytes having a diameter  $\leq 120$   $\mu\text{m}$ . All animals ( $n = 16$ ) were collected from a commercial supplier (Seabait Ltd.) a week prior to the experiments, which took place under controlled conditions in the laboratory. Since birth, animals had been maintained outside under natural photoperiods, so the natural photoperiod at the time of collection was LD 13:11 for A98 and LD 11:13 for W99. After acclimatization, animals were introduced into the actographs (see below) and maintained

under one of two photoperiods, LD 16:8 or LD 8:16, that approximated the photoperiodic extremes between midsummer and midwinter at a latitude of  $55^\circ$  north (where the animals were collected).

Data obtained from the actographic recording were analyzed for two factors: (1) variability in the diel activity of individuals both between photoperiodic treatments and seasons, and (2) overall changes in "strength" of rhythm. Variability in diel activity was visually represented using actograms, while the strength of rhythm was characterized with autocorrelation analysis (Dowse and Ringo, 1989; Palmer *et al.*, 1994; Dutilleul, 1995). This statistic provides a measure of the strength to the rhythm that is not affected by the overall level of activity: the less noisy the data between cycles and the higher the signal, the stronger the rhythm. Since the autocorrelation output is normally distributed around the lag time that most closely matches the period of that particular rhythm, robust parametric statistics can be applied.

The concurrent long-term experiment was carried out for 9 months under constant LD 16:8 and LD 8:16 photoperiods, using animals from the same broodstock that were born naturally at Seabait Ltd. on 16 March 1998 and reared continuously outside under natural photoperiods until the start of these investigations. As with experiments A98 and W99, the animals chosen had about the same weight ( $2.5 \pm 0.1$  g) and sexual maturity (oocyte diameter  $\leq 120$   $\mu\text{m}$ ). All stock animals ( $n = 100$ ) for this experiment were maintained continuously under photoperiods of LD 16:8 or LD 8:16. Such continuous rectilinear photoperiods have previously been used to detect circannual rhythms (Randall *et al.*, 1998; Nisimura and Numata, 2001) without the possibly detrimental effects of inducing a free-running state by using continuous light or darkness. Each month between September 1998 and June 1999, four animals were selected at random from stock aquaria in the laboratory and placed into the artificial burrows of the actograph. Their activity was then recorded for 6 to 7 days under the two photoperiodic treatments, LD 16:8 and LD 8:16. At other times, the animals were maintained under the respective photoperiodic treatments LD 16:8 and LD 8:16 in stock aquaria. Hourly actograph data were summed into 48-h epochs and partitioned into mean monthly actograms. The mean data points were plotted against maximum overall activity that normalized the amplitude between treatments and made direct comparisons possible.

To prevent transient behavioral activity for both short (A98 and W99) and long-term assays, animals were always acclimatized for 7 days under their new photoregimes.

The actograph consisted of two aquaria housed in light-tight photoperiodically controlled chambers. Artificial burrows consisting of lengths of PVC tubing were inserted through the bottoms of these experimental aquaria and connected to infra-red optocouplers that were broken as

soon as any animal emerged. Out-of-burrow activity for all animals was monitored using a data logger. A complete description of the actographic procedures has been published in Last (2003). The aquaria were supplied with recirculating seawater, salinity 34 to 36‰, that had been biologically trickle-filtered and sterilized with UV light; temperatures in the aquaria were maintained constant at  $\approx 16^\circ\text{C}$ . Every 2 days during the middle of the photophase (the light portion of the cycle) the experimental animals were fed coarse trout pellets to and above the maximum that they would consume.

## Results

### *Analysis of locomotor activity in N. virens: Autumn (A98)*

**Actograms.** The mean hourly activity (beam breaks) of all animals over 7 days in the autumn when maintained under LD 16:8 and LD 8:16 photoperiods is shown in Figure 1. Note that these animals are from natural photoperiods of LD 13:11. The main onset of activity under both treatments occurred at 0000 (midnight) GMT immediately after the lights-off signal. All activity was much reduced during the photophase. Thus the type of activity under both treatments can be described as a nocturnal diel pattern of foraging behavior. Under LD 16:8 (Fig. 1a), activity was consistently high for each successive scotophase (the dark portion of the cycle) but with substantial within-treatment variability between individuals, as shown by the standard deviation bars. Under LD 8:16, however (Fig. 1b), the distinction between onset and cessation of activity was less marked except for days 1 and 2. All animals were similarly inactive except during the first scotophase, which showed raised activity levels comparable with those animals under LD 16:8. The mean number of emergence events for animals under LD 16:8 was significantly greater (Student's  $t$  test:  $t = 3.23$ ,  $P < 0.05$ ) for each consecutive 24-h period than for animals under LD 8:16.

**Strength-of-rhythm analysis of activity data.** Autocorrelation analysis showed that animals maintained under LD 8:16 in the autumn have a significantly weaker rhythm of activity than those under LD 16:8 ( $t$  test:  $t = 2.54$ ,  $P < 0.05$ ). This demonstrates that strength of rhythm in the autumn is dependent on the imposed photoperiodic treatment.

### *Analysis of locomotor activity of N. virens: Winter (W99)*

**Actograms.** The mean hourly activity (beam breaks) of all animals over 6 days in the winter when maintained under LD 16:8 and LD 8:16 photoperiods is shown in Figure 2. Note that these animals are from natural photoperiods of LD 11:13. Under both photoperiods, activity was consistently high for each successive scotophase despite the relatively high within-treatment variability between individuals, as

shown by the standard deviation bars. There was no significant difference ( $t$  test:  $t = 2.09$ ,  $P > 0.05$ ) in the number of emergence events for animals under LD 16:8 and animals under LD 8:16 for each consecutive 24-h period. This is in striking contrast to the results recorded 5 months earlier under the same photoperiodic treatments.

**Strength-of-rhythm analysis of activity data.** The strength of the activity rhythm of animals maintained under LD 16:8 was not significantly different ( $t$  test:  $t = 0.65$ ,  $P > 0.1$ ) from those maintained under LD 8:16. This demonstrates that strength of rhythm in the winter is independent of the imposed photoperiodic treatment.

### *Comparative analysis in strength of rhythm: Autumn and winter*

Autocorrelation analysis of rhythm strength is not affected by overall amplitude of activity, and hence a comparative analysis of activity between treatments (LD 16:8 and LD 8:16) and between seasons (A98 and W99) was possible even though overall activity in A98 was higher than in W99. The strength of rhythm of animals maintained under LD 16:8 in the autumn was significantly ( $t$  test:  $t = 2.54$ ,  $P < 0.05$ ) stronger than that of animals under LD 8:16 at this time. No significant difference ( $t$  test:  $t = 0.27$ ,  $P > 0.1$ ) was observed in strength of rhythm in animals maintained under a photoperiod of LD 16:8 in the autumn compared to animals maintained under the same photoperiod in the winter. Similarly, no significant difference ( $t$  test:  $t = 0.65$ ,  $P > 0.1$ ) was observed in strength of rhythm in animals maintained under LD 16:8 or LD 8:16 photoperiods in the winter. Finally the strength of rhythm of animals maintained under the LD 8:16 photoperiod in the winter was significantly ( $t$  test:  $t = 3.29$ ,  $P < 0.01$ ) stronger than that of animals under the LD 8:16 photoperiod in the autumn. In the autumn, the most noticeable between-treatment variation was the much reduced overall activity and strength of rhythm under LD 8:16 compared to LD 16:8. In the winter, no such differences in strength were observed since the strength of rhythm was high in all animals irrespective of photoperiod.

### *Analysis of long-term locomotor activity of N. virens*

The results from each experimental treatment (LD 16:8 and LD 8:16) were pooled to plot a three-dimensional (3D) topographical graph of activity (y-axis) over time (x-axis) and month (secondary x-axis) (Fig. 3a, b). Dashed rectangles beneath the x-axis represent the times of the imposed scotophase. The surface topography represents the mean hourly activity of four animals over the duration of 6 to 8 days every month for 9 months. (Note: these results do not include data from the short-term A98 and W99 experiments).

Figure 3a shows the pattern of locomotor activity under

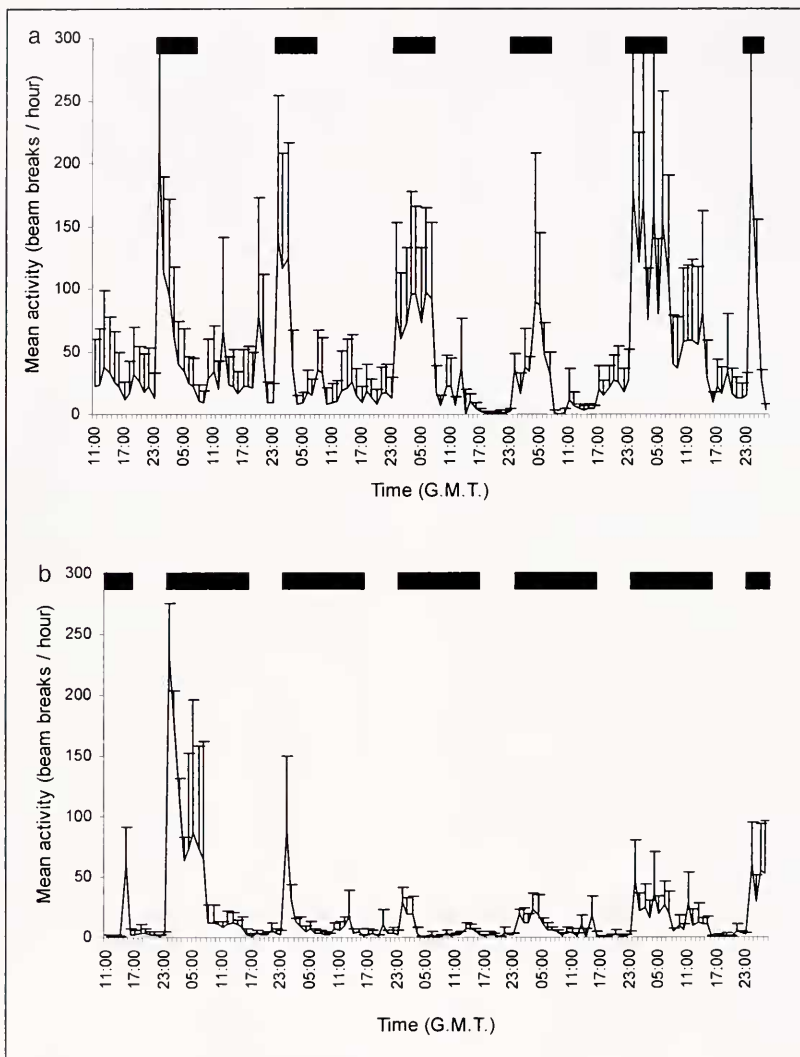


Figure 1. The mean beam breaks/hour (+SD) representative of foraging excursions under different photoperiods in the autumn (A98) for *Nereis virens*, where the black rectangles represent times of artificial scotophase. (a) LD 16:8,  $n = 4$ ; (b) LD 8:16,  $n = 4$ .

LD 16:8 over the duration of the experiment. Most of the activity was restricted to the scotophase, except transiently in September and March, when late photophase activity was also observed. Little difference was seen between mean activities over each successive 48-h period for the duration

of 9 months. Activity was typically initiated around the time of the lights-off signal (steep topographical relief) and continued to be high throughout all scotophases.

Figure 3b shows the pattern of locomotor activity under LD 8:16. Between September and December there was no

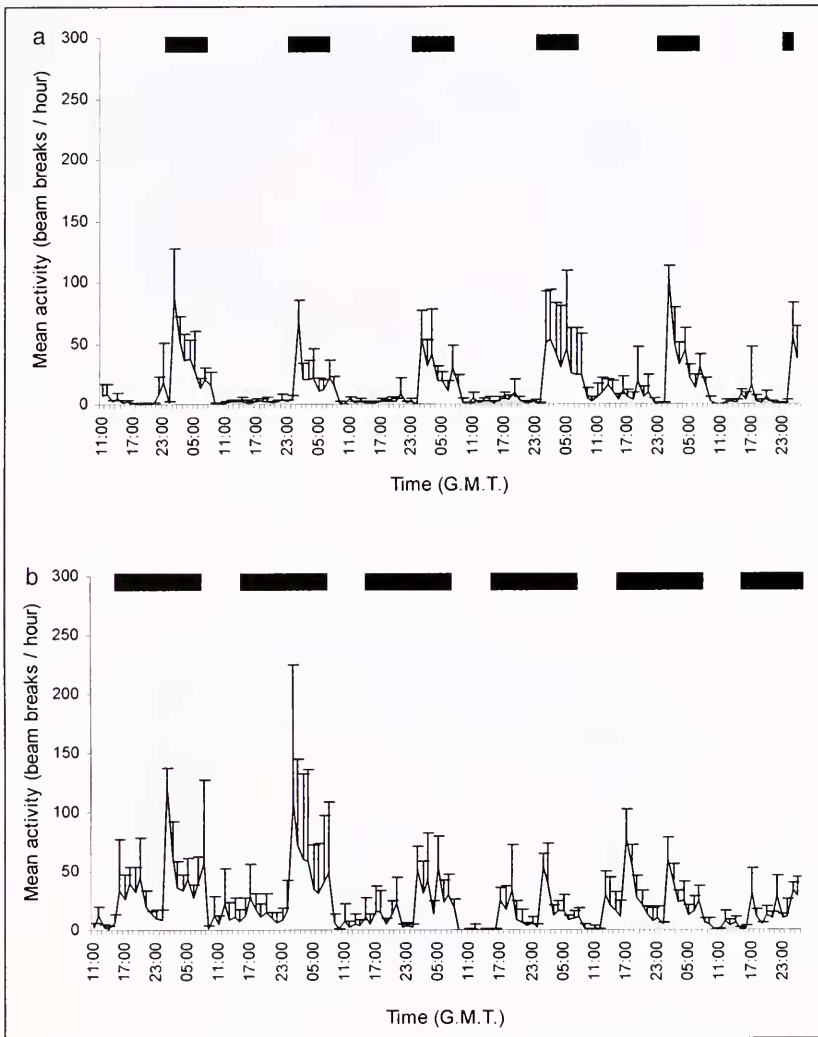
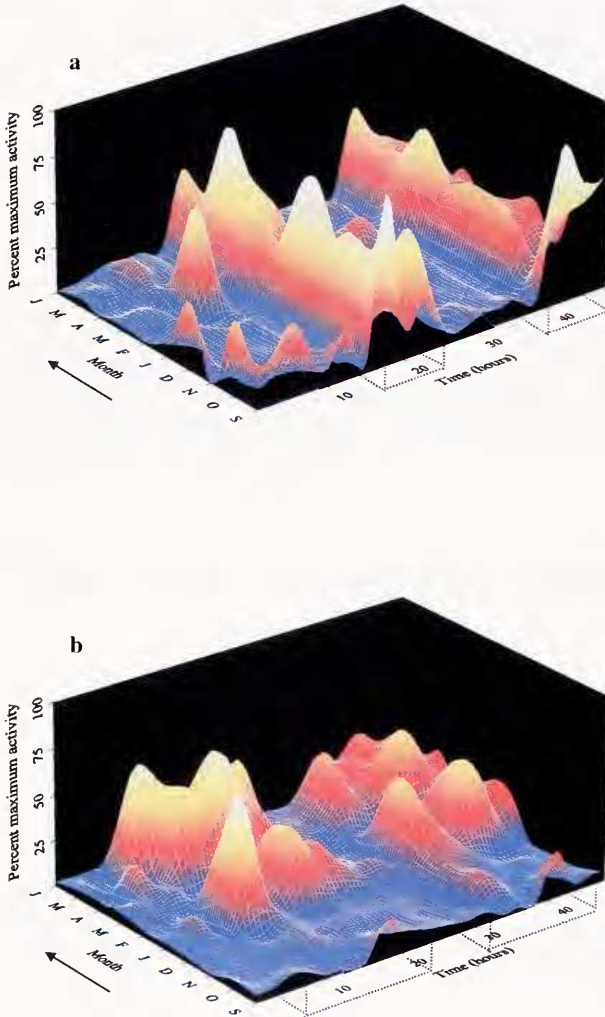


Figure 2. The mean beam breaks/hour (+SD) representative of foraging excursions under different photoperiods in the winter (W99) for *Nereis virens*, where the black rectangles represent times of artificial scotophase. (a) LD 16:8,  $n = 4$ ; (b) LD 8:16,  $n = 4$ .

activity in either the photophase or the scotophase. Nocturnal activity increased dramatically after December, and then continued until termination of the experiment in June. This elevated activity occurred spontaneously and independently of any environmental signal. The major peak of activity was generally seen directly after lights-off, with raised activity

during the rest of the scotophase. Activity during the photophase was low. A perturbation was observed in March, when activity dropped significantly for the duration of the month. This is represented by the trough running the length of the graph from left to right.

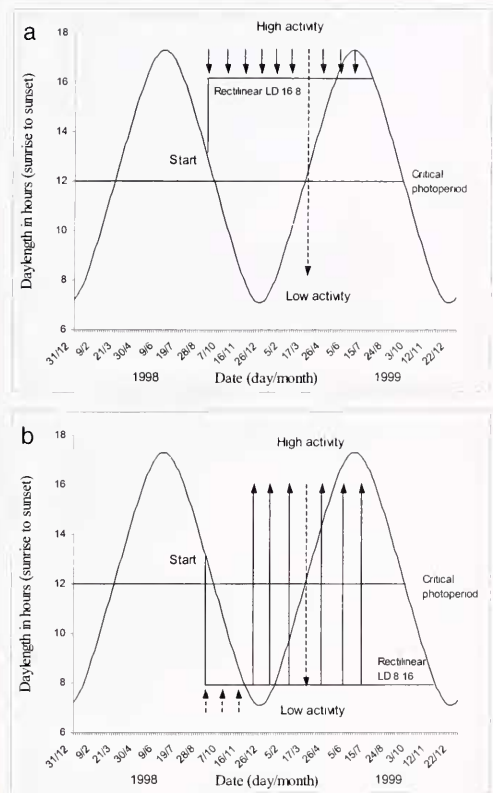
The results from the topographical data for LD 16:8 and



**Figure 3.** Three-dimensional plot showing the topography of mean monthly activity (September to June) in *Nereis virens* under different static photoperiods. Dashed rectangles beneath the x-axis represent times of the scotophase. Data points constituting surface topography number about 7000. (a) LD 16:8,  $n = 4$ : Nearly all activity is confined to the scotophase except for perturbations in September, October, and March. (b) LD 8:16,  $n = 4$ : Note the cessation of activity between September and the middle of December. At the beginning of January, a spontaneous resurgence of activity during the scotophase occurs, which is continuous for the rest of the experiment except for a decline in activity in March.

LD 8:16 are summarized schematically in relation to the natural photoperiod in Figure 4. At the start of the experiment in September, the natural photoperiod was LD 13:11.

All animals placed under the LD 16:8 photoperiod (Fig. 4a) showed strong, nocturnal activity that continued until March, when the natural photoperiod was once again LD



**Figure 4.** Schematic summaries of the results for the static rectilinear 9-month experiment under LD 16:8 (a) and LD 8:16 (b). Ambient day length (sunrise to sunset) is shown over time at latitude 55° N, giving the temporal position of the rectilinear photoperiodic treatments and type of activity in relation to the ambient photoperiods outside the laboratory. Solid arrows indicate where activity in *Nereis virens* is high; dotted arrows indicate where activity is low.

12:12. At this time, additional activity was noted during the later part of the photophase. After March, activity was once again restricted solely to the scotophase. Animals placed under the LD 8:16 photoperiod, however, displayed a quite different activity pattern (Fig. 4b). In these animals, all activity was relatively low for 3 months, during the time when natural photophases are diminishing in the natural habitat (LD <12:>12). By the middle of December, the time when the natural scotophase is at its longest at this latitude (17 h), a spontaneous recovery of activity during the scotophase was observed. This pattern of elevated nocturnal activity continued in the laboratory at a time when the nights in the natural environment were gradually shorten-

ing. At the spring equinox in March, when natural photoperiods were once again LD 12:12, overall activity dropped substantially. From April to June, however, activity was once again elevated.

## Discussion

The results presented here demonstrate that the pattern of locomotor activity expressed by *Nereis virens* is influenced by both the photoperiod to which the animals are subjected and the time of year in which the behavioral assay is conducted. Photoperiod and time of year appear to interact and modulate overall activity patterns, giving rise to variability in the daily nocturnal activity patterns and in the overall strength of the rhythm associated with foraging activity.

Our results also show that the activity of this polychaete is predominantly nocturnal under all photoperiods, irrespective of season. The photophase suppressed activity in animals under both photoperiodic treatments, and we believe that spurious beam breaks during the photophase constitute primarily "within-burrow" rather than "out-of-burrow" activity (Last, 2003). We hypothesize that the conserved nature of this nocturnal response reflects a strong selective advantage as a predator-avoidance mechanism, since feeding excursions for *N. virens* are associated with predation risk from shorebirds and pleuronectenoid fish, which are all well-known diurnal, visual feeders (Thijssen *et al.*, 1974; Carter *et al.*, 1991; Wilson, 1991), and such risk is reduced under cover of darkness.

### Activity in the autumn

Under the LD 16:8 photoperiod in the autumn, the actogram showed a high level of nocturnal activity (Fig. 1a), which is synonymous with a high feeding rate (Last, 2003). In contrast, those animals under LD 8:16 displayed relatively reduced overall activity (Fig. 1b) signifying a low feeding rate. These findings are similar to those of Last and Olive (1999) using semiquantitative, manual methods to assess the degree of foraging activity at a similar time of year.

Photoperiodism necessitates the presence of an underlying endogenous clock. The results of experiment A98 support the presence of such an oscillator in *N. virens*. During the first scotophase, animals under LD 8:16 ceased activity at between 1000 and 1100 GMT (Fig. 1b), 10–11 h after the onset of activity. This was surprising since the lights-on signal for these animals was at 1600 GMT, and inactivity was thus not expected for a further 7–8 h. The animals used in the A98 experiment would have been experiencing natural photoregimes of LD 12:12, suggesting that the observed time of activity cessation was due to the entrainment of past photoperiodic regimes. We hypothesize that the nocturnal activity cessation under LD 8:16 may reflect a

proactive response to the anticipated time of sunrise, therefore providing indirect evidence for an endogenous timer. This response is surprising since these animals had been acclimatized to this photoperiod for a week.

Activity in subsequent scotophases rapidly became bimodal (Fig. 1b) with activity peaks in both early and late scotophases. Similar daily changes in activity under various photoperiods have been recorded for the locomotory activity patterns of the onion fly *Delia antiqua* (Watari and Arai, 1997). When this diurnal fly was subjected to photoperiods in which  $LD >8:<16$ , two peaks of activity were always observed. The first occurred in the early photophase and the second in the late photophase. Under short photoperiods (where  $LD <8:>16$ ), the activity peaks fused as the "window" of diurnal activity became smaller. For this species, it was suggested that the main, late-photophase activity peak was due to a predictive circadian oscillator, whereas the early-photophase peak was solely due to a reactive lights-on response. For the experiment described here using *N. virens*, the response is interpreted as being an adjustment to the new photoperiodic cue, or Zeitgeber, where the observed inactivity at the expected dawn is evidence for transient behavior prior to attaining a steady state in the following scotophases.

Coupling between the environmental Zeitgeber and the endogenous oscillator occurs through entrainment (Aschoff, 1965). The results illustrate that the process of entrainment of an activity rhythm in the autumn experiment depends on the photoperiodic regime. Subjecting animals to a photoperiod of LD 8:16 considerably reduced the strength of the rhythm. We believe that this represents the initial stages of the autumn feeding cessation, which is triggered when the animals pass through a critical photoperiod  $LD_{crit} <12:>12$  (Last *et al.*, 1999), a characteristic physiological response at this time of year. This photoperiodically mediated change in activity provides an interesting result. At a time when the nights were becoming longer and hence, for these nocturnal animals, the "potential" for nocturnal feeding was gradually increasing, all activity became much reduced. It has been demonstrated elsewhere (Last and Olive, 1999) that under a photoperiod of LD 8:16, rates of somatic growth, segmentation, and regeneration all become much reduced and energy is channeled into future reproductive growth through changes in vitellogenesis or vitellin incorporation into the developing oocytes (Rees and Olive, 1999). We regard this as the initiation of a switch from somatic to reproductive growth. The photoperiodic transition therefore appears to trigger a change in physiological state (McNamara and Houston, 1996) from one in which essentially somatic growth processes predominate to one in which acquired resources are redeployed to sexual development. It seems at first surprising that this change in physiological state is not restricted to animals that will breed in the following spring, but occurs in all individuals regardless of age or state of

maturity. A fitness model based on the life history of *N. virens* suggests, however, that when either or both net foraging risk and net foraging gain vary seasonally, a substantial fitness benefit accrues when all members of a mixed-age population respond to environmental signals and reduce foraging at times of maximum risk or minimum benefit (Olive *et al.*, 2000, 2001).

Parallels can be drawn with the Salmonidae, in which temperature and daylength alone do not dictate physiological changes. Bimodality in wild fish will separate animals destined to smolt in the following spring from those that are not (Thorpe *et al.*, 1980; Skilbrei, 1991), a mechanism that has long been known to be photoperiodically induced (Villareal *et al.*, 1988). In *S. salar*, the advancement of smoltification through photoperiodic manipulation has been shown (Duston and Saunders, 1995) to largely reduce the effects of bimodality and has provided evidence that, in the salmonids at least, a coupling between threshold length, photoperiod (and the circadian clock), and time of year (the seasonal/circannual clock) determines bimodal segregation of individuals and maturational development.

#### Activity in the winter

The seasonal modulation of activity in *N. virens* was further exemplified when the same experiments were repeated in the winter. Whereas in the autumn there had been a nearly complete cessation of foraging activity under LD 8:16 (Figs. 1b and 3b), by the winter the amplitude of activity under LD 8:16 (Fig. 2b) had dramatically increased and was high compared to activity in the autumn or under LD 16:8 (Fig. 2a). In addition, the results of autocorrelation analysis showed no significant between-treatment difference in mean strength of rhythm between winter LD 8:16 and LD 16:8. We postulate that, for animals that have not reached some critical state of sexual maturity, the optimum strategy must change as the optimum time for breeding approaches. In these animals, renewed foraging increases the probability that sufficient energy reserves will be accrued for reproduction one year later. Since out-of-burrow foraging by *N. virens* is inevitably associated with predation risk, increased nocturnal activity at this time will maximize exploitation of the feeding time and minimize the risk of predation from diurnal predators. Animals that have reached a critical state of maturity become susceptible to a feedback response from the maturing gametes (Porchet and Cardon, 1976; Golding and Yuwono, 1994). This seasonally mediated change in activity under LD 8:16 occurred independently of photoperiodic treatment, which is suggestive of an endogenous seasonal (and possibly circannual) rhythm of activity or the operation of some long-term interval timer.

Interestingly, the mean peak activity during the first two nights occurred around midnight (Fig. 2b), which cannot readily be explained at this stage. We hypothesize that, as in



the A98 experiment (Fig. 1b), activity onset may have been due to a "memory" of past photoperiodic regimes. The 7-day acclimatization period imposed on these animals under their new photoregime may not have been long enough to prevent transient activity before a new steady state had been reached. The observed "snapshot" of activity under the autumn and winter photoperiodic treatments may be influenced by the photoperiodic history of the animals. In view of these results, a long-term assay under constant photoperiods was deemed the only suitable method for establishing true endogenous behavior without transient manifestations. Under those conditions, any spontaneous changes in activity would imply the effects of a purely endogenous, rather than exogenous, response to an innate underlying seasonal oscillator or interval timer.

#### *Long-term activity patterns in N. virens*

The results shown in Figure 3b demonstrate that a long-term regulator of activity does indeed exist in the polychaete *N. virens*. This endogenous modulation is revealed under a constant LD 8:16 photoperiod, 3 months after the transition through the critical photoperiod  $LD_{crit} < 12 > 12$  previously described by Last *et al.* (1999). The resurgence of activity under this photoperiod was spontaneous and, irrespective of the experimental photoperiod, occurred around the time of the winter solstice. Subjecting animals to a photoperiod of LD 16:8 will result in the negative masking (Aschoff, 1960) of any long-term endogenous oscillators.

Proactive anticipation of the spring equinox has been suggested previously for other nereid polychaetes (Olive and Garwood, 1983; Fong and Pearse, 1992a, b), and the results presented here provide further evidence for the expression of an overt endogenous seasonal clock in the Nereidae. Perturbations in the activity patterns of *N. virens* were observed under both photoperiodic treatments in March. Under LD 16:8, a late photophase peak was observed prior to the scotophase (Figs. 3a and 4a), and under LD 8:16 a marked drop in activity was observed in both the photophase and the scotophase (Figs. 3b and 4b). These perturbations occurred when the animals would, under natural photoperiods, pass through the spring critical photoperiod (where  $LD_{crit} > 12 < 12$ ). The significance of these perturbations is not yet clear, but may indicate a change in the sensitivity of the endogenous pacemaker to the external photoperiod around the time of the spring equinox.

Seasonally overt changes in physiological state and behavior of this long-lived polychaete do not occur solely as a direct response to changes in proximate factors such as temperature and, particularly, daylength. We believe that while seasonally changing daylengths will actively drive changes in physiological state through photoperiodism at certain times of the year, at other times, photoperiod acts as a Zeitgeber to entrain an endogenous seasonal rhythm. Such

a mechanism has also been implicated in another marine invertebrate, *Pisaster ochraceus*. In this sea star, gametogenesis can be shifted by maintaining the animals on a seasonally changing photoperiod regime out-of-phase with ambient photoperiods (Pearse *et al.*, 1986). Like our results, this finding is suggestive of an endogenous annual calendar.

To infer that these results are evidence for a circannual rhythm in *N. virens* would be premature since the observed response could have also been caused by an interval timer measuring a time period of approximately 90 days. Work is in progress to examine the endogenous modulation of activity patterns over the duration of years instead of months. We provide here evidence of a long-term modulation of activity in *N. virens* under fixed photoperiods. This mechanism modulates the response of individuals to the natural photoperiod according to real time and may be an essential adaptive component of the photoperiodically adjusted seasonal cycle of growth and reproduction.

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#### Literature Cited

- Aschoff, J. 1960. Exogenous and endogenous components in circadian rhythms. *Cold Spring Harbor Symp.* 25: 11–28.
- Aschoff, J. 1965. Circadian rhythms in man. *Science* 148: 1427–1432.
- Carter, C. G., D. J. Grove, and D. M. Carter. 1991. Trophic resource partitioning between two coexisting flatfish species off the north coast of Anglesey, North Wales. *Neth. J. Sea Res.* 23: 325–335.
- Dowse, H. B., and J. M. Ringo. 1989. The search for hidden periodicities in biological time-series revisited. *J. Theor. Biol.* 139: 487–515.
- Duston, J., and R. L. Saunders. 1995. Advancing smolting to autumn in age 0+ Atlantic salmon by photoperiod, and long-term performance in sea water. *Aquaculture* 135: 295–309.
- Dufilleul, P. 1995. Rhythms and autocorrelation analysis. *Biol. Rhythm Res.* 26: 173–193.
- Fong, P. P., and J. S. Pearse. 1992a. Evidence for a programmed circannual life cycle modulated by increasing daylengths in *Neanthes limnicola* (Polychaeta: Nereidae) from central California. *Biol. Bull.* 182: 289–297.
- Fong, P. P., and J. S. Pearse. 1992b. Photoperiodic regulation of parturition in the self-fertilising viviparous polychaete *Neanthes limnicola* from central California. *Mar. Biol.* 112: 81–89.
- Garwood, P. R., and P. J. W. Olive. 1982. The influence of photoperiod on oocyte growth and its role in the control of the reproductive cycle of the polychaete *Harmothoe imbricata* (L.). *Int. J. Invertebr. Reprod.* 5: 161–165.

- Golding, D. W., and E. Yuwono. 1994.** Latent capacities for gametogenic cycling in the semelparous invertebrate *Nereis*. *Proc. Natl. Acad. Sci. USA* **91**: 11777–11781.
- Last, K. S. 2003.** An actograph and its use in the study of foraging behaviour in the benthic polychaete, *Nereis virens* Sars. *J. Exp. Mar. Biol. Ecol.* **287**: 237–248.
- Last, K. S., and P. J. W. Olive. 1999.** Photoperiodic control of growth and segment proliferation by *Nereis (Neanthes) virens* Sars in relation to real time and state of maturity. *Mar. Biol.* **134**: 191–199.
- Last, K. S., P. J. W. Olive, and A. Edwards. 1999.** An actographic study of diel activity in the semelparous polychaete *Nereis (Neanthes) virens* Sars in relation to the annual cycle of growth. *Invertebr. Reprod. Dev.* **35**: 141–145.
- Marcus, N. H. 1986.** Population dynamics of marine copepods: the importance of photoperiodism. *Am. Zool.* **26**: 469–477.
- McNamara, J. M., and A. I. Houston. 1996.** State-dependent life-histories. *Nature* **380**: 215–221.
- Nisimura, T., and H. Numata. 2001.** Endogenous timing mechanism controlling the circannual pupation rhythm of the varied carpet beetle *Anthrenus verbasci*. *J. Comp. Physiol. A* **187**: 433–440.
- Olive, P. J. W., and P. R. Garwood. 1983.** The importance of long term endogenous rhythms in the maintenance of reproductive cycles of marine invertebrates—a reappraisal. *Int. J. Invertebr. Reprod.* **6**: 339–347.
- Olive, P. J. W., J. Fletcher, S. Rees, and G. Desrosiers. 1997.** Interactions of environmental temperature with photoperiod in determining age at maturity in a semelparous polychaete *Nereis (Neanthes) virens* Sars. *J. Therm. Biol.* **22**: 489–497.
- Olive, P. J. W., C. Lewis, and V. Beardall. 2000.** Fitness components of seasonal reproduction: an analysis using *Nereis virens* as a life history model. *Oceanol. Acta* **23**: 377–389.
- Olive, P. J. W., C. Lewis, V. Beardall, K. Last, and M. G. Bentley. 2001.** Fitness consequences of seasonal reproduction: experiments on the polychaete *Nereis virens* Sars. Pp. 321–341 in *Environment and Animal Development*, D. Atkinson and M. Thornødyke, eds. Bios Scientific Publishers, Oxford.
- Palmer, J. D., B. G. Williams, and H. B. Dowse. 1994.** The statistical analysis of tidal rhythms: tests of the relative effectiveness of five methods using model simulations and actual data. *Mar. Behav. Physiol.* **24**: 165–182.
- Pearse, J. S., and D. J. Eernisse. 1982.** Photoperiodic regulation of gametogenesis and gonadal growth in the sea star *Pisaster ochraceus*. *Mar. Biol.* **67**: 121–125.
- Pearse, J. S., D. J. Eernisse, V. B. Pearse, and K. A. Beauchamp. 1986.** Photoperiodic regulation of gametogenesis in sea stars, with evidence for an annual calendar independent of fixed daylength. *Am. Zool.* **26**: 417–431.
- Porchet, M., and C. Cardon. 1976.** The inhibitory feed-back mechanism coming from oocyte and acting on brain endocrine activity in Nereidae. *Gen. Comp. Endocrinol.* **30**: 378–390.
- Randall, C. F., N. R. Bromage, J. Duston, and J. Symes. 1998.** Photoperiod induced phase shifts of the endogenous clock controlling reproduction in the rainbow trout: a circannual phase response curve. *J. Reprod. Fertil.* **112**: 399–405.
- Rees, S. W., and P. J. W. Olive. 1999.** Photoperiodic changes influence the incorporation of vitellin yolk protein by oocytes of the semelparous polychaete *Nereis (Neanthes) virens*. *Comp. Biochem. Physiol. A* **123**: 213–220.
- Skilbrei, O. T. 1991.** Importance of threshold length and photoperiod for the development of bimodal length frequency distribution in Atlantic salmon (*Salmo salar*). *Can. J. Fish. Aquat. Sci.* **48**: 2163–2172.
- Tijssen, R., A. J. Lever, and J. Lever. 1974.** Food composition and feeding periodicity of 0-group plaice (*Pleuronectes platessa*) in the tidal area of a sandy beach. *Neth. J. Sea Res.* **8**: 369–377.
- Thorpe, J. E., C. Talbot, and C. A. Villarreal. 1980.** Bimodality in growth and smolting in Atlantic salmon, *Salmo salar* L. *Aquaculture* **28**: 123–132.
- Villarreal, C. A., J. E. Thorpe, and M. S. Miles. 1988.** Influence of photoperiod on growth changes in juvenile Atlantic salmon, *Salmo salar* L. *J. Fish Biol.* **33**: 15–30.
- Watari, Y., and T. Arai. 1997.** Effects of photoperiod and aging on locomotor activity rhythms in the onion fly, *Delia antiqua*. *J. Insect Physiol.* **43**: 567–576.
- Wilson, W. H. 1991.** The importance of epibenthic predation and ice disturbance in a Bay of Fundy mudflat. *Ophelia* **5**: 507–514.